# Impact of atmospheric ammonia on growth, C and N accumulation and photosynthesis of two maize cultivars with different N root supply

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### **ABSTRACT**

Impact of enriched atmospheric  $\operatorname{NH}_3$  in combination with low and high N medium on growth, total C and N accumulation ( $C_{\text{tot}}$ A and  $N_{\text{tot}}$ A) and photosynthetic characteristics of two maize cultivars i.e. SD19 (cult. 1) and NE5 (cult. 2) with low N and N high use efficiency, respectively, was investigated. Plants were exposed to 10 nl/L and 1000 nl/L NH $_3$  fumigation, respectively, for 30 days in open-top chambers (OTCs). Under exposure to the low N medium, increase of the atmospheric NH $_3$  concentration to 1000 nl/L from the ambient level significantly (P < 0.05) increased dry matter (DM) (by 18% in cult. 1 and 14% in cult. 2 respectively),  $C_{\text{tot}}$ A,  $N_{\text{tot}}$ A, net photosynthetic rate ( $P_n$ ), stomatal conductance ( $G_s$ ) and apparent quantum yield (AQY) but decreased intercellular  $CO_2$  concentration ( $C_i$ ) in both cultivars. These effects were more pronounced in cult. 1 as compared to those in cult. 2. In contrast, in the high N solution, enriched atmospheric NH $_3$  led to a decrease in DM,  $C_{\text{tot}}$ A,  $N_{\text{tot}}$ A,  $P_n$ ,  $G_s$  and AQY but an increase in  $C_i$  of cult. 2 only. Dark respiration rate remained unaffected by enrichment of NH $_3$  in each treatment. Therefore, it is concluded that appropriately enriched atmospheric NH $_3$  can improve plant growth of maize by enhancing  $C_{\text{tot}}$ A,  $N_{\text{tot}}$ A, and photosynthesis in the low N medium, especially for low N use efficiency cultivars.

**Keywords**: atmospheric ammonia enrichment; dry matter; carbon accumulation; nitrogen accumulation; photosynthetic characteristic

Among the atmospheric N species, ammonia (NH $_3$ ) is not only an important atmospheric pollutant for semi-natural vegetation, but one of the key N sources for N-deficiency plants in atmosphere. Annual NH $_3$ -N deposition in each of the 4 years 2003–2006 was estimated to increase from 3.0  $\pm$  0.2 kg N/ha year in ambient air, with the NH $_3$  concentration at 0.5 m above the canopy of 0.7  $\mu$ g/m $^3$ , to 50–70 kg N/ha year where annual

average air concentrations were  $70-90~\mu g/m^3$  and concentrations during fumigation were up to  $1600~\mu g/m^3$  (Cape et al. 2008). Factors contributing to this significant increase in atmosphere NH $_3$  concentration include: vicinity to location of significant NH $_3$  emissions, climatic conditions, and agriculture activities. The latter contributes to 50% of the global NH $_3$  emissions (Fangmeier et al. 1994). Maize (*Zea mays* L.) is a staple food

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crop in northern China. This region is usually subjected to increasing atmospheric NH3 emission to 0.30-1.60 mg/m<sup>3</sup> from regular mean air NH<sub>3</sub> concentration of 0.01–0.20 mg/m<sup>3</sup> due to high rate application of NH<sub>3</sub> forming fertilizers (Li et al. 2004). Atmospheric NH<sub>3</sub> is deposited to soil and water, either by dry deposition of NH<sub>3</sub> or by dry and wet deposition of ammonium (NH<sub>4</sub>) (Fangmeier et al. 1994). As a plant nutrient, plants can utilize atmospheric NH3 to improve plant growth and/or production if the mole fraction of NH<sub>2</sub> in the atmosphere is greater than the mole fraction of gaseous NH<sub>3</sub> in the substomatal cavity. Foliar NH<sub>3</sub> uptake by the leaves of Italian ryegrass (Lolium multiflorum) at 0.52 mg/m<sup>3</sup> supplied 47.3% and 35.2% of total plant N at fertilization levels of 100 and 200 mg  $^{15}\mathrm{NO_3^-}\text{-N/kg}$  dry soil, respectively (Fangmeier et al. 1994). It was found that atmospheric NH<sub>3</sub> input is closely correlated with the morphology and metabolism of crops (Bohme et al. 2003, Li et al. 2009).

Exposure of plants to enriched atmospheric NH<sub>3</sub> may result in a significant impact on plant growth, total C and N ( $C_{tot}$  and  $N_{tot}$ ) accumulation as well as photosynthesis, whose responses were dependent on plant species, growth stages and N availability for root uptake (Van der Eerden et al. 1991,1992, Sommer et al. 1993, Tatsuro et al. 2001, Li et al. 2004,2009). Li et al. (2009) concluded that enriched atmospheric NH<sub>3</sub> concentration increased shoot dry matter of wheat (Triticum aestivum L.) in the low N treatment but reduced the plant biomass in the high N treatment. In most cases, exposure to NH<sub>3</sub> generally results in an increase of soluble proteins concentrations in spruce (Piceaabies) trees, N<sub>tot</sub> and chlorophyll concentrations of Scots pine needles as well as photosynthesis of Pinus sylvestris, poplar trees and Douglas fir and dark respiration rate (R<sub>d</sub>) of *Populus euramericana* as long as the tissue is not injured (Van Hove et al. 1989, Van der Eerden et al. 1992, Van der Eerden and Pérez-Soba 1992). Castro et al. (2005) pointed out that atmospheric NH<sub>3</sub> up to 4 μl/L can be regarded as a nutrient for the fast growing of B. oleracea. The concentration at which NH<sub>2</sub> changes from being a nutrient to a toxin is not clear-cut, since NH<sub>3</sub> can still be metabolized when plant growth is already affected (Fangmeier et al. 1994).

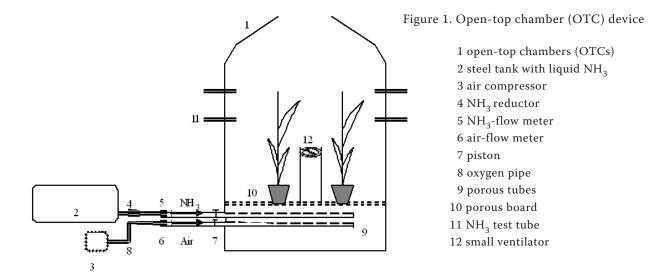
However, above studies mainly focus on forest and grassland vegetation. Much less attention has been paid to the effects of atmospheric NH<sub>3</sub> in agro-ecosystems. Additionally, evaluation of responses of maize crop to varying concentration

of atmospheric  $\operatorname{NH}_3$  are not fully investigated. Keeping in view the above facts, we hypothesize that higher constitutive accumulation of  $C_{\text{tot}}$  and  $N_{\text{tot}}$  and photosynthesis induced by enriched atmospheric  $\operatorname{NH}_3$  may provide a mechanism of the improvement of plant growth of different maize cultivars under two levels of N in solution medium. Therefore, the objective of this study was to examine the growth response and accumulation of  $C_{\text{tot}}$  and  $N_{\text{tot}}$  as well as photosynthetic characteristic parameters of two maize cultivars, with different N use efficiency of plant, exposed to two levels of  $\operatorname{NH}_3$  concentration and N root supply in solution medium for 30 days.

### **MATERIALS AND METHODS**

Plant material and experimental design. Solution culture experiments were conducted at Institute of Soil and Water Conservation, Chinese Academy of Sciences (Yangling, China). Two maize (*Zea mays* L.) cultivars i.e. SD19 (cult. 1) and NE5 (cult. 2) with low N and high N use efficiency, respectively, were supplied for the present experiments.

The open-top chambers (OTCs) used in the experiments (Figure 1) are containers described by Paul and Bert (1993), initially designed for exposing plants to elevated CO<sub>2</sub> concentrations under close to natural conditions, with a square  $1.2 \times$ 1.2 m base and 1.5 m tall perpendicular glass walls topped by glass quadrilaterals inclined towards the centre in an iron frame (total volume circa 3 m<sup>3</sup>). The chambers were each equipped with a fan and an air control system, which included a steel cylinder (inner diameter 600 mm, total length 1800 mm) containing 95% NH<sub>3</sub>. The NH<sub>3</sub> was fed from the bottom into the OTCs through a YQA-441 NH<sub>3</sub> decrement gauge with a pressure range of 0-4 MPa (Shanghai Shuangying Boat Decompressor Manufacture Co., Ltd., Shanghai, China) and Φ8 constant pressure oxygen pipes. The NH<sub>3</sub> flux was measured using an LZB-2 flux meter with anti-corrosion glass rotameter (measuring range 6–60 ml/min, rated working pressure ≤ 1 MPa) (Changzhou Shuangfa Thermal Instrument Co., Ltd., Changzhou, China). In addition, air was fed from the bottom of the OTCs using a ZB-0.10/8 air compressor (air displacement 0.1 m<sup>3</sup>/min, rated air pressure 0.8 MPa) and Φ8 constant pressure oxygen pipes (Shanghai Luodi Air Compressor Co., Ltd., Shanghai, China). The air flux was maintained at 1.7 L/min, as measured by an LZB-2 flux meter



with an anti-corrosion glass rotameter (measuring range 0.25−2.5 m³/h, rated working pressure ≤ 1 MPa) (Changzhou Shuangfa Thermal Instrument Co., Ltd., Changzhou, China).

The temperature, humidity and  $\mathrm{NH_3}$  concentration inside each chamber were regulated by passing air (heated and moistened as appropriate) and  $\mathrm{NH_3}$  through porous pipes at the bottom of the container, while the fan (providing air speed of less than 0.5 m/s) was used to maintain close to uniform distribution of  $\mathrm{NH_3}$  and reduced temperature (if necessary) in the chamber. The temperature in the chambers was monitored to verify that the temperature was the same in the chamber before and after  $\mathrm{NH_3}$  was supplied, and thus that the effects of varying the  $\mathrm{NH_3}$  concentration on the growth parameters or C and N metabolism of plants were not confounded by variations in temperature.

The NH<sub>3</sub> concentration in each chamber was measured four times per day (at 8:00, 11:00, 14:00 and 17:00 h) by a GTL-C indoor air detector equipped with a pH618 test pen (NH<sub>3</sub> testing precision, ± 0.01 mg/m³) (Shanghai Minyi Electron Co., Ltd., Shanghai, China) mounted on a tripod placed in the centre of the chamber before the gas was supplied. On each sampling occasion, 5 ml of NH<sub>3</sub> test reagent was extracted by an injector and injected into a glass bottle for sampling. The glass bottle was immediately plugged and connected to the instrument. During the tests, the flux was adjusted to 2 L/min and the exhaust time was controlled by the auto-timing device. When the sampling time was complete, the glass bottle was removed, unplugged, the reagent in the glass bottle was poured into the test cup and the test pen was inserted into the cup to measure the  $\rm NH_3$  concentration. Throughout the entire growth period, from 8:00–18:00 h every day, the  $\rm NH_3$  concentration in the OTCs used for the background, ambient (control) and elevated  $\rm NH_3$  treatments were maintained precisely at 10 nl/L and 1000 nl/L, respectively, by continuously supplying  $\rm NH_3$  and air at appropriate ratios.

**Plant growth.** Seeds of two maize cultivars were surface-sterilized in  $10\%~H_2O_2$  solution for 15~min. After rinsing in distilled water, seeds were imbibed for 12~h and then sown in porcelain trays containing quartz sand. Seeds were germinated in the dark under 23°C covering with clean wet filter papers. When the root grew to the length of 2-4~cm, the seedling was transferred and fixed in the holes of styrofoam boards by using absorbent cotton in deionized water in plastic trays in the growth chamber under the conditions with 25/18°C of average day/night temperature, 60-70% relative humidity, and 350~µmol/m²/s light intensity and 16/8~h of light/dark regime. The solution was replaced once a day and aerated continuously.

PVC (polyvinyl chloride) pots with a volume of 3534 cm³ (inner diameter 15 cm, height 20 cm) containing 3.5 L nutrient solution were used for plant growth. Ammonia fumigation and N treatments were initially proceeded on the  $3^{\rm rd}$  day after the seedling transferred into OTCs at their three-leaf stage. The experimental design contained three factors which were NH³ atmospheric concentration (at two levels: 10 nl/L, air background concentration and 1000 nl/L, high NH³ concentration), N rate in medium (at two levels: 1/3 and 1/9 N concentration of complete nutrition solution i.e. 5.00 and 1.67 mmol/L nitrate) and maize cultivar (the two cultivars mentioned above), respectively, in a complete factorial design experiment with eight

treatments, and five replications per treatment. The 40 pots (one plant per pot) were randomly placed in four OTCs, and in order to reduce the experimental error the pots were exchanged in the four OTCs every 7 days. The low and high N rate in medium were served by 1/9 and 1/3 strength of the complete Hoagland nutrient solution, respectively. Desired N concentrations were maintained by irrigating sufficiently with new solution. The nutrient solution was amended with higher alcohol to make emulsion in order to restrain NH<sub>3</sub> exchange between two phase of gas and liquid. The pH of solution was adjusted to 6.2 ( $\pm$  0.1). The top of pot was placed on a board with small holes for plants fixation, and the space between plants and holes were sealed with wax. The solution was aerated without NH3 twelve hours a day. The plants of two maize cultivars were maintained for 30-day growth period under each treatment. The whole experiment was carried out twice independently under the same environmental conditions. Data presented here are means of five replicates of the two experiments (n = 10).

Measurements of dry matter and concentrations of total C and total N. Shoots of five plants in five pots of representing each cultivar and each of the treatments were collected from each chamber on the 30<sup>th</sup> day of N and NH<sub>3</sub> fumigation treatments (from 11:00 to 13:00 h), respectively. The samples were cleaned with distilled water, and were dried in a forced-ventilation oven at 65°C until constant dry weight. The dried samples were ground to pass a 1 mm screen for total C  $(C_{tot})$  and total N  $(N_{tot})$  assay. Concentrations of  $C_{tot}$  and  $N_{tot}$  were measured by potassium dichromate (K2Cr2O2) volume method and the Kjeldahl method using an automatic N analyzer (Gerhardt Vapodest 5), respectively. Their total C and N accumulation (C<sub>tot</sub>A and N<sub>tot</sub>A) based on the aboveground dry matter were calculated by multiplying their C and N content and dry matter, respectively.

Determination of photosynthetic parameters. The second completely developed leaf from the top of sample plant on the  $30^{\rm th}$  day of NH $_3$  fumigation were measured from 9:00 to 11:00 h, respectively, using LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). The two leaves of one plant were determined and this was repeated in three replicates. The determination covered net photosynthetic rate ( $P_n$ , μmol  $CO_2/m^2/s$ ), intercele lular  $CO_2$  concentration ( $C_i$ , μmol/mol), stomatal conductance ( $G_s$ , mol/m $^2/s$ ) under the conditions of photosynthetically active radiation (PAR) of 1200 μmol/m $^2/s$  and ambient  $CO_2$  concentra-

tion of 360  $\mu$ mol/mol. Apparent quantum yield (AQY, mol CO<sub>2</sub>/mol) and dark respiration rate (R<sub>d</sub>,  $\mu$ mol CO<sub>2</sub>/m²/s) were computed referring to the index model (1) of light response of maize under the setting PAR values of 2000, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50 and 0  $\mu$ mol/m²/s in turn and ambient CO<sub>2</sub> concentration of 360  $\mu$ mol/mol condition (Guo et al. 2005).

$$P_n = P_{\text{max}} (1 - e^{-\frac{AQY PAR}{P_{\text{max}}}}) - |R_d|$$
 (1)

Where:  $P_n$  represents leaf net photosynthetic rate under different PAR, and  $P_{\text{max}}$  indicates maximum net photosynthetic rate (µmol  $CO_2/m^2/s$ ).

Data statistical analysis. All were subjected to the analysis of variance (ANOVA) with the SAS software package. Appropriate standard errors of the means (S.E.) were calculated for presentation with table and bar diagram. The significance of the treatment effect was determined using the *F*-test, and to determine the significance of the means, least significant differences (*LSD*) were estimated at 5% probability level, and Duncan's multiple range test was used for comparing treatments within two or three factors combinations.

# **RESULTS AND DISCUSSION**

**Plant growth**. Plants can absorb moderate atmospheric NH<sub>3</sub> as source of N, and can thus positively benefit in term of plant growth and biomass production. Atmospheric NH<sub>3</sub> can enter the leaves of higher plants almost exclusively through the stomata and is dissolved in the water film of the mesophyll cells to form NH<sub>4</sub> as long as the ambient NH<sub>3</sub> concentrations exceed the mesophyll concentration (compensation point) (Fangmeier et al. 1994). Since NH<sub>3</sub> uptake by leaves occurred directly following stomatal conductance, the plant response to atmospheric  $\mathrm{NH}_3$  is dependent on environment factors, including crop cultivar and soil moisture availability (Rogers and Aneja 1980), internal CO2 and NH3 concentration, and plant water and fertilizer availability (Tatsuro et al. 2001). At the ambient NH<sub>3</sub> concentrations, its uptake by stem may have limited role in N-nutrition of higher plants. Enhanced growth was observed in many experiments within a reasonable range NH<sub>3</sub> concentrations, not approaching toxic level (Fangmeier et al. 1994). Enriched NH<sub>3</sub> concentrations can contribute to N-nutrition of plants, as was shown by Faller (1972) and Li et al. (2009).

 $Table \ 1. \ \textit{F-} values \ of \ the \ effects \ of \ NH_3 \ level \ (NH_3), \ cultivar \ (Cv), \ N \ level \ (N) \ and \ their interactions \ on \ dry \ matter production \ (DM), \ total \ C \ accumulation \ (C_{tot}A) \ and \ total \ N \ accumulation \ (N_{tot}A) \$ 

Variation (g/plant)	N	Cv	$\mathrm{NH}_3$	N × Cv	$\mathrm{NH_3} \times \mathrm{N}$	$Cv \times NH_3$	$N \times Cv \times NH_3$
DM	176.55***	138.78***	0.97	22.46***	55.94***	2.70	0.40
$C_{tot}A$	4478.17***	2701.51***	78.84***	396.97***	388.39***	12.01**	22.70***
$N_{tot}A$	7718.18***	109.58***	1088.65***	292.19***	72.12***	78.82***	93.57***

 $<sup>^*</sup>P < 0.05; ^{**}P < 0.01; ^{***}P < 0.001$ 

The current study supports the above conclusion as is evident from the positive effects of enriched atmospheric NH3 concentration on plant growth in the low N (LN) solution. Fumigation of 1000 nl/L NH<sub>3</sub> greaterly increased DM production in SD19 (cult. 1) with low N use efficiency (NUE) (by 18%) than that in NE5 (cult. 2) with high NUE (by 14%) above the ambient concentration, which better counteract the negative effect of LN supply on shoot DM (Figure 1). Moreover, the single factor of N level and cultivar together with the interactions of N level and cultivar or NH3 level had significant effects on DM production only (Table 1). Nitrate-deprived plants and low N use efficient cultivar can benefit from the atmospheric N source, since shoot biomass production recovered from nitrate deprivation (Castro et al. 2005). Atmospheric NH<sub>3</sub> at a concentration of 4 µmo1/L was therefore able to replace nitrate as nutrient to a considerable extent. This is in agreement with previous findings (Castro et al. 2005) and theoretical calculations on the possible contribution of NH3 to the N budget of plants (Clement et al. 1997). In the high N (HN) solution, however, enriched atmospheric NH<sub>3</sub> reduced the DM production of cult. 2 (by 16%) with high NUE and non-significant effects of enriched atmospheric NH<sub>3</sub> on DM were found in cult. 1 with low NUE (P > 0.05) (Figure 2). Thereby, NH<sub>3</sub> assimilation under enriched atmospheric NH3 under LN medium can improve plant growth. The canopy will respond to elevated atmospheric NH<sub>3</sub> concentration first, especially for the cultivar with low NUE in most natural environment with N limitation for plant biomass production (Fangmeier et al. 1994).

Accumulation of total C and total N ( $C_{tot}A$  and  $N_{tot}A$ ). The absorption and utilization of NH<sub>3</sub> supplied to the plants was evaluated by calculating  $C_{tot}A$  and  $N_{tot}A$ , respectively. Ammonia (NH<sub>3</sub>) uptake may cause an autocatalytic increase of additional NH<sub>3</sub> flux into the leaves by inducing stomatal opening via the internal  $CO_2$  level (Van der Eerden et al. 1992). Long-term exposure of

plants to moderate atmospheric NH<sub>3</sub> concentration may stimulate increased C and N assimilation in plant, which affects the internal C and N status of the plant (Castro et al. 2005). In Calluna vulgaris (heather) and Deschampsia flexuosa (hair-grass), the N content increased four-fold after exposure to 0.1 mg/m<sup>3</sup> NH<sub>3</sub> for 38 weeks. Varied increases of N<sub>tot</sub>A induced by enriched atmospheric NH<sub>3</sub> were found in other plants such as conifers, tomato (Lycopersicon esculentum), Arnica montana L. and Viola canina L. (Van der Eerden et al. 1991, 1992, Dueck and Elderson 1992, Clement et al. 1997, Li et al. 2009). Our study showed that enriched atmospheric NH<sub>3</sub> concentration increased C<sub>tot</sub>A and N<sub>tot</sub>A in both cultivars in the LN solution, relative to counterparts exposed to the ambient concentration. The above increases in biomass C<sub>tot</sub>A and N<sub>tot</sub>A were greater for cult. 1 (by 16% and 38%, respectively) than that for cult. 2 (by 12% and 32%, respectively). In contrast, in the

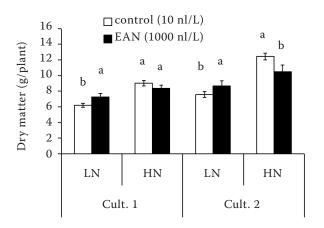


Figure 2. Differential effects of enriched atmospheric  $\mathrm{NH_3}$  on dry matter production (g/plant) of SD19 (cult. 1) and NE5 (cult. 2) in the low and high N (LN and HN) medium. Data represent mean of ten replicates (n=10). At the top of each column, different letters indicate significant differences between enriched atmospheric  $\mathrm{NH_3}$  (EAN-1000 nl/L) and control treatment (10 nl/L) with the same cultivar and N level

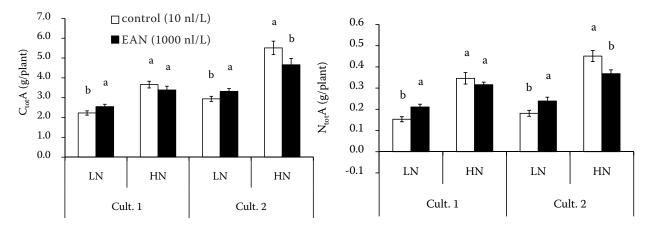


Figure 3. Differential effects of enriched atmospheric NH $_3$  on total C accumulation ( $C_{\rm tot}A$ ) (g/plant) of SD19 (cult. 1) and NE5 (cult. 2) in the low and high N (LN and HN) medium. Data represent mean of ten replicates (n=10). At the top of each column, different letters indicate significant differences between enriched atmospheric NH $_3$  (EAN-1000 nl/L) and control treatment (10 nl/L) with the same cultivar and N level

Figure 4. Differential effects of enriched atmospheric NH $_3$  on total N accumulation (N $_{\rm tot}$ A) (g/plant) of SD19 (cult. 1) and NE5 (cult. 2) in the low and high N (LN and HN) medium. Data represent mean of ten replicates (n=10). At the top of each column, different letters indicate significant differences between enriched atmospheric NH $_3$  (EAN-1000 nl/L) and control treatment (10 nl/L) with the same cultivar and N level

HN solution, enriched atmospheric  $\mathrm{NH_3}$  more decreased  $\mathrm{C_{tot}}\mathrm{A}$  and  $\mathrm{N_{tot}}\mathrm{A}$  of cult. 2 (15% and 18%, respectively) than those of cult. 1 (non-significant) (Figures 2–3). Additionally, the effects of  $\mathrm{NH_3}$  level, cultivar, N level and their interactions on  $\mathrm{C_{tot}}\mathrm{A}$  and  $\mathrm{N_{tot}}\mathrm{A}$  were all signifiaent (Table 1). Such, enhancement of  $\mathrm{C_{tot}}\mathrm{A}$  and  $\mathrm{N_{tot}}\mathrm{A}$  may be significant for plants in natural environments if the  $\mathrm{NH_3}$  concentration rises, since N is a limit-

ing factor for plant biomass production in most natural habitats, especially for low NUE cultivars (Fangmeier et al. 1994, Li et al. 2009).

**Photosynthesis.** Increases of  $CO_2$  assimilation together with net photosynthetic rate  $(P_n)$  and apparent quantum yield (AQY) enhancement resulted in lower intercellular  $CO_2$  concentration  $(C_i)$  of plants. The modulation of  $CO_2$  concentration in plants might be increase  $G_s$  (stomatal conductance),

Table 2. Differential effects of enriched atmospheric  $NH_3$  on photosynthesis parameters of SD19 (cult. 1) and NE5 (cult. 2) in the low and high N medium

Treatment		$P_n \atop (\mu \text{mol CO}_2/\text{m}^2/\text{s})$	$C_i \ (\mu mol/mol)$	$G_s$ (mol/m <sup>2</sup> /s)	AQY (mol CO <sub>2</sub> /mol)	$R_d$ (µmol $CO_2/m^2/s$ )	
Low N (1.67 mmol/L) combined with different NH <sub>3</sub> concentration							
Cult. 1	control	$16.18 \pm 0.89^{b}$	$269.2 \pm 10.6^{a}$	$0.139 \pm 0.016^{b}$	$0.078 \pm 0.003^{b}$	$1.273 \pm 0.180^{a}$	
	EAN	$20.63 \pm 1.55^{a}$	$195.2 \pm 10.3^{\rm b}$	$0.193 \pm 0.018^{a}$	$0.095 \pm 0.004^{a}$	$1.290 \pm 0.220^{a}$	
Cult. 2	control	$25.02 \pm 2.52^{b}$	$204.2 \pm 8.3^{a}$	$0.175 \pm 0.014^{\rm b}$	$0.093 \pm 0.004^{\rm b}$	$1.638 \pm 0.120^{a}$	
	EAN	$28.70 \pm 2.33^{a}$	$163.4 \pm 7.9^{b}$	$0.225 \pm 0.016^a$	$0.108 \pm 0.005^{a}$	$1.636 \pm 0.180^{a}$	
High N (5.00 mmol/L) combined with different NH <sub>3</sub> concentration							
Cult. 1	control	$24.02 \pm 1.12^{a}$	$179.3 \pm 8.8^{a}$	$0.205 \pm 0.014^{a}$	$0.099 \pm 0.002^{a}$	$1.312 \pm 0.159^{a}$	
	EAN	$22.96 \pm 1.03^{a}$	$188.3 \pm 8.7^{a}$	$0.183 \pm 0.014^{a}$	$0.091 \pm 0.003^{a}$	$1.327 \pm 0.156^{a}$	
Cult. 2	control	$38.24 \pm 2.22^{a}$	$130.0 \pm 7.5^{a}$	$0.255 \pm 0.018^a$	$0.123 \pm 0.002^{a}$	$1.750 \pm 0.220^{a}$	
	EAN	$33.17 \pm 2.03^{b}$	$153.0 \pm 8.3^{\rm b}$	$0.212 \pm 0.016^{\mathrm{b}}$	$0.105 \pm 0.005^{\mathrm{b}}$	$1.744 \pm 0.250^{a}$	

 $C_i$  – intercellular  $CO_2$  concentration; control – ambient  $NH_3$  concentration (10 nl/L);  $G_s$  – stomatal conductance; AQY – apparent quantum yield; EAN – enriched atmospheric  $NH_3$  concentration (1000 nl/L);  $P_n$  – net photosynthetic rate;  $R_d$  – dark respiration rate. Data represent mean of ten replicates (n = 10). Mean values followed by different letters within each column indicate significant differences at P < 0.05 between enriched atmospheric  $NH_3$  fumigation and control treatment with the same cultivar and N supply

Table 3. *F*-values of the effects of NH<sub>3</sub> level (NH<sub>3</sub>), cultivar (Cv), N level (N) and their interactions on photosynthesis parameters

Variation	N	Cv	NH <sub>3</sub>	N × Cv	$NH_3 \times N$	$Cv \times NH_3$	$N \times Cv \times NH_3$
$P_n (\mu \text{mol CO}_2/\text{m}^2/\text{s})$	445.20***	980.25***	2.29	32.44***	116.64***	13.11**	6.02*
$C_i$ (µmol/mol)	10106.2***	10416.3***	2121.1***	49.81***	6664.2***	690.9***	112.6***
$G_s  (\text{mol/m}^2/\text{s})$	2036.6***	2908.9***	204.75***	16.29**	3844.7***	84.1***	38.9***
AQY (mol CO <sub>2</sub> /mol)	677.60***	1524.60***	12.60**	35.00***	1177.40***	50.40***	22.40***
$R_d (\mu mol CO_2/m^2/s)$	16.54**	123.50***	0.01	14.43**	0.01	0.01	0.04

 $C_i$  – intercellular  $CO_2$  concentration; AQY – apparent quantum yield;  $G_s$  – stomatal conductance;  $P_n$  – net photosynthetic rate;  $R_d$  – dark respiration rate. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001

which is beneficial for transmition and absorption of atmospheric NH<sub>3</sub> (Fangmeier et al. 1994). Van Hove et al. (1989), Van der Eerden et al. (1992) and Van der Eerden and Pe'rez-Soba (1992) pointed out that increased NH3 concentration enhanced P<sub>n</sub> of *Pinus sylvestris*, poplar trees and Douglas fir, respectively. However, unaffected results in P, of sunflower (Helianthus annnus L.) and Acacia auriculaeform were obtained by Berger et al. (1986) and Zhao et al. (2003), respectively. In this study, the authors stated that enriched NH3 concentration increased  $P_n$ ,  $G_s$  and AQY but decreased  $C_i$ in both cultivars in the LN treatment, especially for low NUE cultivar (cult. 1). On the contrary, in the HN treatment, enriched atmospheric NH<sub>2</sub> decreased P<sub>n</sub>, G<sub>s</sub>, AQY but increased C<sub>i</sub> of cult. 2 only. Dark respiration rate (R<sub>d</sub>) of both cultivars was not affected by increased atmospheric NH<sub>3</sub> irrespective of N treatment (Table 2). In addition, the signifiaent effects of NH<sub>3</sub> level, cultivar, N level and their interactions on  $C_i$ ,  $G_s$  and AQY were all obtained. With respect for  $P_n$ , non-significant effect of NH<sub>3</sub> level was merely calculated (P > 0.05). Nitrogen level, cultivar and their interaction had a significant effect on R<sub>d</sub> only (Table 3). Similar result was drawn out by Van Hove et al. (1989) in *Populus euramericana* under 50–100 μg/ m<sup>3</sup> NH<sub>3</sub> concentration for 6–8 weeks. However, increase by 76% of  $R_{\rm d}$  in Populus euramericana was found under 240 μg/m<sup>3</sup> NH<sub>3</sub> concentration for three months. Thus, the boundary factors for efficient effects of NH<sub>3</sub> fumigation on R<sub>d</sub> need to further clarify. The results here show that elevated atmospheric NH<sub>3</sub> concentration played a distinct role in the modulation of these photosynthesis parameters except for R<sub>d</sub> of the plants, whose responses were dependent on crop cultivar and N supply in the medium (Fangmeier et al. 1994). These consistencies of photosynthesis physiological parameters change can be useful evaluation index

for plant growth under increased atmospheric  $\mathrm{NH_3}$  environment. Such atmospheric  $\mathrm{NH_3}$  absorption by the plant canopy might offset the N deficiency from root absorption, which showed the stronger use ability of atmospheric  $\mathrm{NH_3}$ . The possible mechanism might be as following: relative deficiency of N nutrition of plant is beneficial for absorption from atmospheric  $\mathrm{NH_3}$  through leaves and synthesis of Rubisco which results in the increase of  $\mathrm{P}_n$  (Zhao et al. 2003). Under high N medium, excessive  $\mathrm{NH_3}$  concentrations in cell possibly restrain Rubisco activity which reduces  $\mathrm{P}_n$  of plants (Fangmeier et al. 1994).

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